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ORIGINAL ARTICLE

Nutritive Assessment of Spent Seaweed and Fermented Spent Seaweed *Sargassum wightii*

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ABSTRACT
In the present study spent seaweeds biomasses of Sargassum wightii, and fermented spent Sargassum wightii were investigated for its proximate composition viz., Carbohydrate (28.76%) , Ash (27%), Moisture (11.05%), crude fiber (16.05%), lipid content (1.33 %) and Protein (7.07%) were observed in spent Sargassum wightii. Crude protein (15.1%) significantly increases and decrease in the levels of carbohydrate (29.1%) and crude lipid (0.49%) were observed in fermented spent Sargassum. Such that anti nutritional factors, total polyphenol, tannin and saponin were decreased in fermented Sargassum. Likewise, mineral contents such as Mg (60.57±0.43), Ca (18.33±0.2), and Fe (0.54±0.01), increased in fermentation spent Sargassum powder expressed in ppm. From the results reveals that the spent seaweeds has greater potential to be used as fuel, fodder and fertilizer which is an alternate source of seaweed based industries.
Keywords*: Seaweed, spent seaweed Sargassum, Yeast fermentation, nutritional level, anti-nutritional factor.*

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INTRODUCTION

Seaweed based industrial discharge contains marine organic wastes which remains unexplored. Appropriate use of these marine organic wastes for valuable products enhances the prosperity of seaweed farmers as well as it minimizes the environmental discharge [30]. Seaweeds are the only source for industrial phycocolloids (agar, carrageenan, alginate and fucoidan) and they are being recognized as potential source of natural pigments [49, 45, 46]. Seaweeds are divided into three major groups as Red (Rhodophyceae), Brown (Phaeophyceae) and Green (Chlorophyceae), which constitutes 50-70% carbohydrates on dry weight basis [48]. The Red algae contain either agar or carrageenan as chief carbohydrates and whereas Brown algae contains alginate, mannitol, laminarin or fucoidan. These water extractable phycocolloids have been utilized in an expansive scope of industrial applications as thickening, gelling or stabilizing agents [20, 13].

Traditionally, seaweed biomass is used for extracting phycocolloids, fertilizers, as ingredients of food, feed and cosmetics etc. [22, 10, 36, 37]. In addition seaweeds have recently been recognised as a source of natural pigments for various food and cosmetic applications [32, 45, 46]. Usually, shade-dried biomass of seaweeds is subjected for extraction of phycocolloids [37].

Seaweeds have many bioactive compounds like pigments, sulfated polysaccharides such as agar, carrageenan and alginates, that are used for various industrial applications [7, 26].

Mostly seaweed yields five-carbon and six-carbon sugars on hydrolysis by acid or alkali. In order to convert polysaccharides to monosugars, an effective pretreatment process is necessary. Mild acid treatment was found to be effective on hydrolysis of polysaccharides at a particular temperature [6, 21, 25]. Saccharification and hydrolysis are essential for bioconversion of substrate.

Hence, in this study, the proximate and mineral composition of the spent seaweeds and fermented spent seaweed *sargassum wightii,* collected from the agropyte industry were investigated.

MATERIAL AND METHODS

Experimental design. This study used completely randomized design (RAL) with 3 treatments and 3 replicates. The treatments of the research are as follows:

A = fermentation of Spent *Sargassum* powder with yeast *S. cerevisiae*

B = Spent *Sargassum* powder

D = *Sargassum* Powder (control).

Fermentation.

The fermentation process was performed by the method of Felix & Brindo, [14, 15] with some modifications. The dried seaweed powder to seawater in the ratio of 1:9 (seaweed: seawater) was taken in the fermenter vessel. 10 mL of *S. cerevisiae* was inoculated at a concentration of 3.10 x 10^8. The sugar substrate, dextrose was added at the rate of 5% w/v of the base material. pH of the fermented seaweeds dropped from 7.0 to 4.0 on the 3rdday. The fermentation was carried out till the pH reached at 4.00. The fermented *Sargassum* was collected from the fermenter and dried in a hot air oven at 60^{o}C for 2 days. The fermented *Sargassum* is then used for proximate analysis.

Proximate composition analysis

Estimation of Moisture

Moisture content of *Sargassum wightii* was determined according to the method described by AOAC [1] with modifications. Samples (2 g) were put in a petridish and dried in a hot air oven at 105 °C until constant weights were obtained.

Estimation of Ash Content

Ash content of *Sargassum wightii* was determined according to the method described by AOAC [1] with slight modifications. Dried samples obtained from the moisture content analysis were burnt and ashed in a muffle furnace (Kasba PID-964) at 525 °C overnight.

Estimation of Crude Fiber

Crude fiber was determined by sequential extraction of seaweed samples with 1.25% H_2SO4 and 1.25% NaOH using the fibre-bag as a container. For drying and ashing, the crucible with sample was dried in an oven for 5 hours at 105 °C and ashed in the muffle furnace (Kasba PID-964) at 525°C overnight. The weight of crucible with sample after drying and ashing was recorded and the fiber content was calculated [1].

Phytochemical analysis

Total Phenolic content

The amount of total phenolic content was determined with Folin and Ciocalteu's reagent according to the method of Singleton and Rossi, 1965 with Gallic acid as the standard. Briefly 0.1 ml of sample extract was mixed with 1 ml of Folin and Ciocalteu's reagent (1:2 with water) was added and kept at room temperature for 5 min and then 1 ml of 7% sodium carbonate solution was added to the reaction mixture and incubated at room temperature for 90 minutes. The colour developed was read at 750 nm.

Estimation of Carbohydrate

The total carbohydrate was estimated by phenol-sulphuric acid method by Dubois *et al*. [11]. The carbohydrate content was calculated by referring standard D-glucose and the results are expressed in percentage.

Estimation of Lipid

The lipid was estimated by using chloroform-methanol mixture as described by Folch *et al*., [16]. 10 mg of dried powder sample was taken in a test tube and 5 ml of chloroform- methanol mixture (2:1) was added. The mixture was then incubated at room temperature for 24 hrs followed by filtration using a filter paper. The filtrate was collected in a 10 ml pre weighed beaker, and kept on a hot plate. The initial weight of the empty beaker and the beaker with the residue and was calculated to know the weight of lipid present in the sample.

Estimation of Protein

The protein content was estimated by Biurette method followed by Raymont *et al*., [38]. To 5 mg of dried powdered sample 1ml of distilled water followed by 4ml of biurette reagent was added and the sample was incubated for 30 minutes under room temperature. Then the mixture was centrifuged at 4000 rpm for 10 minutes. The supernatant solution was collected and the optical density was measured at 540 nm.

Micronutrient Estimation

The samples for micronutrient estimation were prepared using muffle furnace and elements were analysed through Atomic Absorption Spectrophotometer (AAS; Shimandzu AA 6200, Scientific Instruments Inc. Columbia, USA).

RESULTS AND DISCUSSION

Fermentation process can breakdown complex compounds such as proteins, carbohydrates, lipids and other organic materials into simpler compounds. Fermentation products can lead to improvement in the nutritional value, and alter basic properties of the product such as improved digestibility and preferred taste and flavor [50]. In the present study, fermentation of spent *Sargassum* powder with yeast *Saccharomyces cerevisiae* resulted in highest protein content ($p < 0.05$). The protein content of fermented *Sargassum* increased from 7.5 to 10.1% compared with crude *Sargassum* powder. This result was similar with previous findings in other plant-based ingredients such as cassava [2], sesame seed meal [29], palm kernel meal [31], and soybean meal [28, 51]). It is well established that protein-rich aqua feed diet is growth promoters [23, 40]. Significant increases in protein contents of fermented *Sargassum* over the spent and fresh *Sargassum* which are highly appealing to speculate the growth promoting factors of the acquafeed. It has been reported that macroalgae can be incorporated as protein sources into the diets of poultry, pigs, cattle, sheep, and rabbits Stephen and Maria [44].

Fermented Spent *Sargassum* powder experienced a significant reduction ($p < 0.05$) in lipid contents, in which the lipid gradually decreased from 1.33% (Sargassum fresh) to 0.49% (Fermented) . However, lipid contents recorded in the present study were higher than those reported in *Sargassum polycystum*, *Padina australis,* and *Turbinaria conoides* [41], and in *Sargassum* sp. [47]. High lipid content foods shows negative effects such as oxidation and produce unpleasant odour.

A significant decrease in carbohydrate contents was also observed in fermented spent *Sargassum* powder (29.1%) and Crude *Sargassum* (50.7%) (Figure 3). This result may be rationalized as these two compounds were the source of energy for the maintenance and production of Yeast. The energy was released into the air as an energy loss. Kusumaningrum *et al*., [24] reported that the decrease of lipid content was due to richness of glucose content in *Sargassum* which induce growth of yeast biomass, resulting in increased production of lipase enzymes to overhaul rough lipids. Decreased carbohydrate contents may be assumed a decrease in crude fibre from *Sargassum* powder, since crude fibre is part of unstable carbohydrates, which can be separated by a nitrogen-free extract (BETN) comprising mainly starch, by analysis simple chemistry [19]. Decreased crude fiber content is caused by the reshuffling of complex substances in *Sargassum* into simpler compounds performed by *S. cerevisiae.* Starch represents a low cost and easily available feed ingredient and thus, the percentage of starch source is desired to be increased in aquafeed. However, the ability of certain fish species to hydrolyze (digest) complex carbohydrates is limited due to weak amylolytic activity in their digestive tracts. Starch digestion decreases as the proportion of dietary starch is increased [17, 5].

Seaweeds in India and from other parts of the world are recognized to contain high amount of total carbohydrates [32, 36, 39].Generally spent biomass showed significant decrease in the total carbohydrate and cellulose content in the samples after extraction

One of the major growth promoting factors in fish diet is protein. Protein is required to maintain normal body functions, and the deficiency of this nutrient can affect the protein synthesis and lead to the reduction in weight and other symptoms in fish, while carbohydrates are an excellent source of energy and carbon in feed formulations [8]. They can be easily distinguished from the other energy-yielding nutrients in terms of their abundance and low price. The dietary inclusion level and appropriate source of carbohydrate are decided based on a protein sparing without any adverse effect on growth and physiology of the fish.

Physiochemical parameters

The ferment spent *Sargassum* powder showed a distinct trend in moisture contents, which is significantly increased compared to that of non fermented spent and crude seaweed 11.5% to 26.2% this is due to translocation of nutrients and other organic matter loss by *Sacchromycess sp.* on fermentation.

Ash contents of fermented spent and non-fermented spent *Sargassum* powder found in the level of 27-34% .Increased ash contents in fermented spent *Sargassum* powder were due to elevated mineral availability of the substrate and breakdown of organic components by fermenting microorganisms of the spent *Sargassum* powder [34]. The ash content of the *Sargassum* powder often correlates with the amount of mineral content in the *Sargassum* powder Dani *et al*., [9]. Ash content is the indication of the amount of mineral elements present in the bioprotein produced after the fermentation process. The results were in consistent with the finding of Tamayo & Rosario, 2014 in *Sargassum* sp. (27.11%) and Sanchez-Machado *et al*., 2004 in *Laminaria ochroleuca* (29.47%). However, ash contents found in this study were much lower than that reported in *S. polycystum* (38%) [27].

Decreased crude fiber content in fermented spent *Sargassum* is caused by the reshuffling of complex substances in crude *Sargassum* into simpler compounds performed by *S. cerevisiae* 16.05 to 2.1%. There are other reports showed similar decrease in fiber content of shrimp head silage meal [33], fermented

duck weed meal from 11.0 % to 7.5 % [3], yeast fermented water hyacinth from 19.0 % to 16.2 % [12] and fermented grass pea seed meal (from 9.6 % to 10 % reduced to 4.9 – 6.5 %). Seaweeds are the cheapest protein sources but their utilization is limited by the presence of high amount of crude fiber which can be eliminated by fermentation process [14]. The fiber contents of fermented *U. lactuca* showed drastic reduction (from 19.61 % to 2.01 % [15].

Significant differences ($p < 0.05$) in tannin contents were recorded among fermented spent seaweed and non-fermented spent *Sargassum* powder. Tannin content (mg g^{-1}) of *Sargassum* powder ranged from 5.50± 0.024 to 3.20 ±0.43 mg g^{-1} shown in Table 2. Significant differences in the tannin content were seen among fermented *Sargassum* powder. The reduction of tannin may be as a result of an increase in the production of alpha-galactosidase by the microorganisms used as starter culture during fermentation. Schons *et al*., [42] reported that fermentation and enzyme and combination of fermentation-enzyme treatments were effective in diminishing tannin and phytate in sorghum flour.

The study revealed that total polyphenol contents were significantly lower in fermented spent *Sargassum* powder than in crude *Sargassum* powder. Total polyphenol contents calculated as catechin in grains of fermented *Sargassum* powder was 3.20 mg catechin/g d.m. while in crude *Sargassum* powder was 5.50 mg catechin/g d.m shown in Table 2. However, total polyphenol contents found in this study were lower compared to *S. classifolium* (1.08 mg g-1), *S. binderi* (1.14 mg g-1), and *S. dublicatum* (1.82 mg g-1) Bambang *et al*., [4]. Variation in total polyphenols content might be due to the different election of reference standards used in each study.

Minerals

Macromineral contents of fermented spent seaweed and non-fermented spent *Sargassum* powder are shown in Table 3. High Mg, Ca concentrations ($p < 0.0\ 5$) were found in *S. cerevisiae* fermented spent *Sargassum* powder while no differences of Na, Zn, Cu, Cd and K contents were observed in *Sargassum* . Significant effects of fermented spent seaweed ($p < 0.05$) were also recorded in Fe content. The macromineral contents of fermented *Sargassum* powder were higher than those found in *S. wightii* . This may be due to break down of soluble minerals into media during fermentation. In addition, fermenting microorganisms might have used it for its metabolic activities as reported by Osman, [35]. Thus, Ca and Fe contents of fermenting *Sargassum* powder could be an indication that certain organisms utilize them for their growth and metabolism Hasan [17]. Minerals in many plant-based feed ingredients may change their chemical form during and after processing such as fermentation, or interaction with other compounds. Hence, the solubilities of the mineral can increase or decrease depending on the type of processing method. However, due to the low protein content of *Sargassum* powder, the interaction between minerals and proteins may not occur [41].

Table :1 : Proximate composition analysis

Proximate composition	***Sargassum wightii***	***Spent Sargassum***	***Fermented Sargassum***
Moisture	11.5	11.4	26.2
Carbohydrate %	50.7	45.3	29.1
Total ash %	27	27	42
Crude fiber %	16.05	12.2	2.1
Total lipid %	1.33	1.02	0.49
Total protein %	7.07	8.1	15.1

Data are expressed as mean±SE, standard error; values indicate no significant difference ($p > 0.05$).

Table.2 Anti-nutritional contents of fermented and non-fermented spent S*argassum* powder

Anti-nutritions	***Sargassum wightii***	***Spent Sargassum***	***Fermented Sargassum***
Total phenolic (mg g-1)	0.90±0.06	0.72±0.04	0.60±0.05
Total Tannins (mg g-1)	5.50± 0.024	3.21±	3.20 ±0.43
Saponin(mg g-1)	**1.52±0.05**	**1.32±0.04**	**1.21±0.04**

Data are expressed as mean±SE, standard error; values indicate no significant difference ($p > 0.05$).

Table 3: Mineral contents of fermented and non-fermented Spent *Sargassum* powder

Elements	*Sargassum wightii*	*Spent Sargassum*	*Fermented spent Sargassum*
Nitrogen	5500±0.72	5400±0.71	7500±0.72
Calcium	17.71±0.34	17.71±0.34	18.33±0.2
Copper	0.03±0.01	0.02±0.01	0.03±0.01
Cadmium	0.02±0.01	0.01±0.01	0.02±0.01
Iron	0.49±0.01	0.46±0.01	0.54±0.01
Magnesium	58.57±0.02	56.57±0.03	60.57±0.43
Potassium	16.81±0.26	15.61±0.26	16.61±0.31
Sodium	9.47±0.27	9.37±0.27	9.41±0.23
Zinc	0.39±0.01	0.37±0.01	0.39±0.02

Data are expressed as mean±SE, standard error; no significant difference ($p > 0.05$).

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